EXHIBIT III

ELITRA.009A

PATENT

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

| | | |
|---|---|---|
| Applicant | : | Forsyth et al. |
| Appl. No. | : | 09/741,669 |
| Filed | : | December 19, 2000 |
| For | : | GENES IDENTIFIED AS REQUIRED FOR PROLIFERATION OF E. COLI |
| Examiner | : | Frank Lu |
| Group Art Unit | : | 1634 |

DECLARATION UNDER 37 C.F.R. § 1.132

**Mail Stop AF**
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

I, R. Allyn Forsyth, declare as follows:

1. I am one of the inventors of the subject matter claimed in the above-referenced patent application.

2. I am familiar with the specification of the present patent application, including the methods described therein and the details of the working examples that are described therein.

3. I have extensive experience in the field of microbial genetics and molecular biology as evidenced by my attached curriculum vitae (Exhibit A)

4. This Declaration is being submitted to demonstrate antisense nucleic acids complementary to a portion of the *E. coli yid*C gene, including SEQ ID NO: 60, inhibit the proliferation of *E. coli*. This Declaration also demonstrates that antisense nucleic acids complementary to a portion of the *Staphylococcus aureus yid*C gene, which were identified substantially as described in the present specification, inhibit the proliferation of *Staphylococcus aureus*.

5. As described in the specification of the present application, and discussed in more detail below, by identifying antisense nucleic acids which inhibit proliferation a number of proliferation-required genes as well as their proliferation-required gene products were identified

in *E. coli*. Among the inhibitory antisense nucleic acids discovered from *E. coli* was the antisense nucleic acid with the Clone ID No. E1M10000118B05. This clone is listed as an inhibitory antisense nucleic acid in Table 1 of the present application and has been given the Sequence Identification Number of SEQ ID NO: 60. The nucleotide sequence of this inhibitory antisense nucleic acid is present in the Sequence Listing of the present application as SEQ ID NO: 60.

6. Antisense nucleic acids capable of inhibiting the activity or level of a gene product encoded by a proliferation-required gene, including the inhibitory antisense nucleic acid having the nucleotide sequence of SEQ ID NO: 60, were identified prior to filing the present patent application using the procedure described in Examples 1 and 2 of this patent application. This procedure is also described below.

a. A library of nucleic acid sequences derived from the *E. coli* chromosome was generated by shearing the *E. coli* chromosome. In particular, random fragments of *E. coli* genomic DNA were generated by DNAse I digestion, made blunt-ended with T4 polymerase, and cloned into the multiple cloning site (MCS) of the inducible expression vectors, pLEX5BA and pLEX5BA-3'.

b. Both pLEX5BA and pLEX5BA-3' are expression vectors which contain a *lac* promoter/operator sequence upstream of the multiple cloning site (MCS) such that the transcription of any nucleic acid fragment that is inserted into the MCS can be induced by adding IPTG to the culture. pLEX5BA-3' is derived from pLEX5BA but differs in that is also includes a T7 transcriptional terminator downstream of the MCS. This terminator prevents transcriptional read through thus allowing only the nucleic acid fragment inserted into the multiple cloning site to be transcribed. Each of these vectors also contains antibiotic resistance genes, such as the ampicillin resistance gene, for use in plasmid selection and maintenance.

c. The restriction site that was used for cloning the sheared genomic fragments into the expression vectors was the *Sma*I site. *Sma*I is a restriction enzyme which cuts DNA leaving blunt ends. Since the genomic fragments were also blunt ended, these fragments were inserted into the expression vector in either a forward orientation (such that the sense strand of the fragment was transcribed) or a reverse orientation (such that the antisense strand of the fragment was transcribed).

d. The library of expression vectors containing the genomic fragments was then transformed into *E. coli* and cultures were grown overnight in the presence of carbenicillin. Plasmids from the overnight cultures were harvested, purified and then re-transformed into *E. coli*. Resulting transformants were plated on LB agar supplemented with carbenicillin thereby generating plates having approximately 500 colonies each. Single colonies were subjected to robotic picking, arrayed into wells of 384 well culture dishes containing liquid LB supplemented with carbenicillin and then incubated for 16 hours at room temperature.

e. To study the effect of transcription of the genomic inserts on the proliferation of cells plated on solid medium, $10^2$, $10^3$, $10^4$, $10^5$, $10^6$, $10^7$ and $10^8$ fold dilutions of each overnight culture were prepared. Aliquots of from 0.5 to 3 µl of these dilutions were spotted on selective agar plates with or without 1 mM IPTG. After overnight incubation, the plates were compared to assess the sensitivity of the clones to IPTG. Clones that grew similarly at all dilutions on both media were scored as a negative and were no longer considered. Clones that grew on the non-IPTG-containing medium but failed to grow at the same serial dilution on medium containing 1 mM IPTG were given a score based on the differential. For example, if the clone grew at a serial dilution of $10^4$ on the IPTG-containing plate and grew at a serial dilution of $10^8$ on the non-IPTG-containing plate, then the corresponding clone received a score of "4" representing the log difference in growth observed.

f. To study the effects of transcriptional induction in liquid medium, growth curves were carried out by back diluting overnight cultures 1:200 into fresh media with or without IPTG and measuring the $OD_{450}$ every 30 minutes (min).

g. Of the numerous clones tested, some were identified as a containing nucleic acid sequences that inhibited *E. coli* proliferation after IPTG induction. Plasmid DNA was recovered from each of these clones then sequenced to determine the number of unique inhibitory sequences that were expressed.

h. The sequence of each unique inhibitory nucleic acid was then used to determine the full-length sequence of the *E. coli* gene corresponding to the insert sequence. In particular, identification of the *E. coli* gene sequence was performed by comparing the sequence of the unique inhibitory nucleic acid to known *E. coli* sequences in GenBank using BLAST version 1.4 or version 2.0.6 as described in Example 3 of the present application.

7. Using the method described above, at least 93 of the inhibitory nucleic acids were found to be unique antisense sequences complementary to at least a portion of the sense strand of an *E. coli* gene. One of the inhibitory antisense nucleic acids discovered during this screen was found to be complementary to a portion of the *E. coli* gene *yid*C. Expression of this antisense nucleic acid resulted in a reduction in cellular proliferation such that a dose of cells 100 times greater than the number of cells required to form a colony on a plate lacking IPTG was required to form a colony on a plate containing 1 mM IPTG. This effect is indicated by the score of "2" that is listed in the column entitled "score" for plate well number B5 (highlighted in yellow) in Exhibit B. Since the plate was numbered E1M10000118, the clone in well B5 was given the Clone ID Number E1M10000B05 (B05). This is the Clone ID Number that corresponds to SEQ ID NO: 60 in TABLE I of the present patent application. As described in the Sequence Listing of the present patent application, the antisense nucleic acid of SEQ ID NO: 60 has a nucleotide sequence of 92 bases that is completely contained within the coding region of the *E. coli yid*C gene.

8. In a second screening experiment, which was conducted using methods substantially identical to those described above, a second antisense nucleic, which was found to inhibit *E. coli* proliferation, was identified as being complementary to a least a portion of the *yid*C gene. This 326 bp antisense nucleic acid was given the Clone ID No. E1M10000288G07 (G07). As with SEQ ID

NO: 60, this new antisense clone inhibited *E. coli* proliferation by a score of "2," which means that a dose of cells 100 times greater than the number of cells required to form a colony on a plate lacking IPTG was required to form a colony on a plate containing 1 mM IPTG. (See Exhibit C, yellow highlighting). Amplification of the plasmid sequence using primers flanking the insert produced a band or the expected size (326 bp) demonstrating that the G07 antisense nucleic acid was present in the cell. (Exhibit C). When cells containing G07 antisense were inoculated onto IPTG plates in sufficient concentration to exhibit visible growth, only a very small colony was formed. This additional phenotypic feature is indicated by the letter "t" in the column entitled "phenotype" in Exhibit D.

9. The length of the G07 inhibitory antisense nucleic acid was found to be 326 nucleotides. Upon comparison of the G07 nucleotide sequence with the known sequence of the *E. coli* genome, it was found that G07 maps to the region between 81.5 and 84.5 minutes of the *E. coli* chromosome. Upon further analysis it was determined that G07 overlaps 157 nucleotides of the 3'-end of the *yid*C coding region.

10. A comparison of the locations of antisense clones B05 (SEQ ID NO: 60) and G07 with respect to the *E. coli yid*C gene showed that each of these antisense nucleic acids are complementary to separate regions of the *yid*C gene separated by approximately 800 base pairs. As such, clones B05 (SEQ ID NO: 60) and G07 are non-overlapping inhibitory antisense nucleic acids which are complementary to different regions of the *yid*C gene.

11. Antisense nucleic acids complementary to the *yid*C gene of organisms other than *E. coli* inhibit proliferation of those organisms. We recently completed a screen of approximately 315,000 *Staphylococcus aureus* clones to identify antisense nucleic acids capable of inhibiting the expression of proliferation-required genes. One of the many inhibitory antisense nucleic acids that was identified was an antisense nucleic acid complementary to at least a portion of the *yid*C gene from *Staphylococcus aureus* (the *yid*C gene is also known as SAV2090 in *S. aureus*).

12. The screen for inhibitory antisense nucleic acids from *S. aureus* was preformed using essentially the methods described in the present application for *E. coli*. The details of this procedure are described below.

a. *Staphylococcus aureus* genomic DNA was sheared as described in the above procedure except that in some instances the enzyme used to digest the DNA was *Sau*3A. Fragments up to 800 bp were size selected using gel electrophoresis and then cloned into the MCS of the expression vector pXylT5-P15a.

b. pXylT5-P15a contains a T5 promoter and a xylose operator sequence upstream of the multiple cloning site (MCS) arranged such that the transcription of any nucleic acid fragment that is inserted into the MCS can be induced by adding the sugar xylose to the culture. pXylT5-P15a also includes a T7 transcriptional terminator downstream of the MCS. This terminator prevents transcriptional read through thus allowing only the nucleic acid fragment inserted into the multiple cloning site to be transcribed.

c. Genomic fragments were cloned into the MCS of pXylT5-P15a without respect to orientation as described in the above procedure. Thus, if the fragment was inserted into the cloning site in a forward orientation, the sense strand of the fragment would be transcribed. In contrast, if the fragment was inserted in a reverse orientation, the antisense strand of the fragment would be transcribed.

d. The library of expression vectors containing the genomic fragments was transformed into electrocompetent *E. coli* strain XL1-Blue MRF' and plated on LB medium supplemented with carbenicillin. Resulting colonies numbering 5 x $10^5$ or greater were scraped and combined, and were then subjected to plasmid purification.

e. The purified library was transformed into electrocompetent *Staphylococcus aureus* RN4220. Resulting transformants were plated on agar containing LB + 0.2% glucose (LBG medium) supplemented with chloramphenicol thereby generating plates having approximately 500 colonies each. Single colonies were subjected to robotic picking, arrayed into wells of 384 well culture dishes containing liquid LBG supplemented with chloramphenicol and then incubated for 16 hours at 37°C.

f. To study the effect of transcriptional induction on solid medium, overnight cultures were serially diluted as described above. Aliquots of from 0.5 to 3 µl of these dilutions were spotted on selective agar plates with or without 2% xylose. After overnight incubation, the plates were compared to assess the sensitivity of the clones to 2% xylose. Clones were scored for growth inhibition in the presence of 2% xylose as described above.

g. Of the 315,000 clones tested, about 4000 were identified as a containing nucleic acid sequences that inhibited *S. aureus* proliferation after xylose induction. Plasmid DNA was recovered from each of these clones then sequenced to determine the number of unique inhibitory sequences that were expressed.

h. The sequence of each unique inhibitory nucleic acid was then used to determine the full-length sequence of the *S. aureus* gene corresponding to the insert sequence. In particular, identification of the *S. aureus* gene sequence was performed by comparing the sequence of the unique inhibitory nucleic acid to known *S. aureus* sequences in GenBank using BLAST.

13. Using the method described above, at least 2100 of the inhibitory nucleic acids were found to be unique antisense sequences complementary to at least a portion of the sense strand of a *S. aureus* gene. Several of the inhibitory antisense nucleic acids discovered during this screen were found to be complementary to a portion of the *S aureus* gene SAV2090 (see Exhibit E). The polypeptide produced by the *S. aureus* SAV2090 gene was found to include the highly evolutionarily conserved YidC region (as shown in Exhibit F), and thus, SAV2090 was identified as the *S. aureus* counterpart of the *yid*C gene in *E. coli*.

14. Although a number of antisense clones complementary to the SAV2090 gene were obtained, only a single antisense nucleic acid, S1M10000048E09 (S1-843A), was selected for

further study (see Exhibit G). First, the inhibitory ability of the S1-843A antisense was further characterized by determining the effect of the expression of S1-843A on the growth of *S. aureus* in broth culture for a range of inducer (xylose) concentrations. In a second study, the $IC_{50}$ for xylose-mediated antisense expression was calculated from a dose response curve that was generated by determining the percentage of proliferation inhibition of cells treated with increasing concentrations of xylose as compared with untreated control cells. The methods, which were used to generate these data and which are described below, are essentially the same as the procedures described in Examples 1 and 9 of the present patent application.

a. To study the effects of transcriptional induction in liquid medium, growth curves were carried out by back diluting logarithmic cultures of *S. aureus* to an $OD_{600}$ of 0.0002 using fresh media with varying concentrations of xylose and measuring the $OD_{600}$ every 10 minutes over the course of twelve hours. In particular, *S. aureus* containing the above-described S1-843A antisense expression construct was used to test the effect of the induction anti-*yid*C antisense expression on *S. aureus* proliferation at fifteen different xylose concentrations ranging from 0 to 100 mM. The results of this experiment are displayed in Exhibit H.

b. To determine the $IC_{50}$ for xylose-mediated S1-843A antisense expression, dose response curves were generated by monitoring the decrease in proliferation of *S. aureus* in response to increasing xylose concentration. In particular, the pXylT5-P15a vector containing S1-843A in an antisense orientation was introduced into *S. aureus* and isolates having the S1-843A expression construct were grown overnight in broth culture. The overnight culture was diluted 1:200 into fresh medium with or without xylose then distributed among the wells of a 96 well microtiter plate in 200 µl aliquots. In particular, xylose was present in the medium at 100 mM, 75 mM, 50 mM, 37.5 mM, 25 mM, 18.75 mM, 12.5 mM, 9.38 mM, 6.25 mM, 4.69 mM, 3.13 mM, 2.34 mM, 1.56 mM, 1.17 mM and 0 mM. Cultures were grown in quadruplicate for each xylose concentration. Additionally, control cells were grown in quadruplicate without xylose. When the control cells (cultures without xylose) reached an $OD_{600}$ of 0.1, the percent growth (relative to the control culture) for each of the xylose containing cultures was plotted against the log concentrations of xylose to produce a growth inhibitory dose response curve for xylose-mediated S1-843A antisense expression. The concentration of xylose that inhibits cell growth to 50% ($IC_{50}$) as compared to the 0 mM xylose control (0% growth inhibition) was then calculated from the curve. The results of this experiment are displayed in Exhibit I.

15. The growth curves displayed in Exhibit H plot the optical density of cultures of *S. aureus*, which harbor the S1-843A antisense nucleic acid under the control of a xylose inducible expression vector, over a twelve hour time course. Each curve represents the proliferation of a culture grown in the presence of xylose ranging in concentration from 0 to 100 mM. These growth curves show that *S. aureus* grown in the presence of high xylose concentrations proliferated much less rapidly over the twelve hour time course than those cells exposed to little or no xylose. In general, as the concentration of xylose was increased, *S. aureus* proliferation decreased.

16. The dose response curve displayed in Exhibit I illustrates that expression of the S1-843A antisense nucleic acid induced with approximately 7.13 mM xylose is sufficient to inhibit *S. aureus* proliferation by about 50%. At a concentration of approximately 16.0 mM xylose, *S. aureus* proliferation is inhibited by about 90%.

17. I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful, false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or patent issuing therefrom.

Dated: August 8, 2003 By: R. Allyn Forsyth

R. Allyn Forsyth

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*Curriculum vitae*

# Roger "Allyn" Forsyth


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San Diego, CA 92121
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## Education:

1989 **B.S. in Biology**
University of California at Los Angeles

1998 **Ph. D. in molecular biology** conferred jointly by
San Diego State University and the
University of California at San Diego.

Delayed degree two years to allow patent prosecution

## Management and corporate leadership accomplishments

- Participated in securing first round funding for start-up company.
- Assisted in licensing discovery method from university
- Responsible for meeting major stage two funding milestones (patent filings, personnel recruitment, equipment acquisition, and scientific proof of concept).
- Responsible for strategic development plans, budgets, FTE and equipment allocation.
- Appointed to corporate Joint Research Committees
- Responsible for successful technology transfer for corporate partners.
- Managed as many as 7 direct reports while coordinating the efforts of over a dozen researchers, scientists and engineers.
- Recruited, trained, performed yearly evaluations and maintained high retention rates for direct reports over a 4 year period in an HTS environment.

## Summary of Scientific and Research skills

- Experienced in assay development, primary HTS, compound profiling.
- Established microbiologist with demonstrated creative molecular biology approaches in a variety of fungal, gram-positive and gram-negative microorganisms.
- Strong skills in technology adaptation in the field of genomics.
- Creative ability to "industrialize" micro and molecular techniques by adapting protocols to 384 well formats and/or high density arrays.
- Comfortable with custom robotic system design, optics and data integration.
- Experienced with bioinformatic tools, analysis and computing platforms (Unix, Vax, PC, Mac), design of Access databases, Excel macros and other data tools.

**Experience:**

2001-Present **Scientific Group Leader at Elitra Pharmaceuticals**

Team leader of an interdisciplinary group developing Elitra's Target Array technology from proof of concept to production level compound screening. The Target Array presently provides the relative sensitization and significance with which over 200 essential S. aureus genes respond to tested compounds simultaneously. I designed and oversaw development of custom robotics platform, the biological strategy for building, implementing and ultimately the transfer of the technology to corporate partners

I have continued development of target-based assays for a collaborator. 10 assays against various essential genes were delivered in a 1-year period.

2000-2001 **Senior Scientist at Elitra Pharmaceuticals.**

Led a team developing cell based target assays in bacteria. Industrialized the process of assay development allowing for parallel processing of multiple targets while collecting "high content" data over time (cell growth at altered levels of target protein, response of cell to dozens of metabolic inhibitors, target mRNA levels etc.). Strategy allowed for prioritization of hundreds of targets and delivery of over a dozen assays (in all major cellular pathways) for collaborators in an 8-month period. Responsible for technology transfer of assay methodologies to collaborators and in managing transitions to HTS.

Directed the establishment of a BioRepository containing over 10,000 engineered strains and plasmids. Data on all fungal, bacterial and plasmid entities is maintained on a customized relational database.

Established and executed novel HTS in 384 formats allowing parallel testing of many targets against more than 20,000 samples per day. Directed secondary screening and profiling of compounds against panels of bacteria.

1998 - 2000 **Research Scientist II / Scientific Cofounder at Elitra Pharmaceuticals (formerly Rajyabiotics)**

Managed a team of 4 - 7 Research Associates engaged in an essential gene discovery program. Program successfully screened more than 6 pathogens during this time and has filed patents on over 3000 genes to date. Responsible for the industrialization of Elitra's core gene discovery process by evaluating, acquiring and integrating robotic equipment. Initiated and managed collaborations to develop new equipment and software to increase efficiency.

Managed and industrialized the company media lab. Increased media production to an excess of 1500 units per day (petri plates, 96 and 384 microtiter plates) and maintained general support of a lab of 30 research personnel while decreasing to 1 FTE. Achieved goals through

custom automation and integrated tracking and ordering systems.

- Developed core protocols for processing gram-negative and gram-positive bacterial organisms using patented gene discovery technique.
- Team processed up to 1,500,000 clones /month as compared to previous efforts of 20,000 /month resulting in discovery rates as high as 300 genes per month.
- Set up databases and tracking systems.

1997 - 1998 **Research Scientist / Scientific Cofounder at RajyaBiotics Pharmaceuticals**

As a scientific co-founder of the company I had multiple roles. I assisted in achieving milestones for seed funding (Series A and B), interviewed and recruited scientific personnel (5 Ph.D.s, 8 RAs), prepared patent and other intellectual data for the essential gene discovery technique, and aided in the design and equipment choices of 6,000 sq ft of laboratory space. The company was based on an essential gene discovery technology that I co-invented and helped license from SDSU.

- Developed key protocols and molecular tools including core vectors and primers, genomic library protocols, screening and validation methodologies.
- Devised approaches to study individual essential genes using inverse PCR strategies or random fractionation of the target genes of interest.

Identified key mechanisms of antisense RNA inhibition in gram-negative organisms.

1992 - 1997 **Consultant**

Macromolecular Structural Analysis Resources Center.
Responsibilities included organizing and presenting workshops to California State University subscribers in San Francisco, Pomona and Fullerton, on the use of GCG and STADEN programs on VAX and UNIX platforms. Consulting with users to ensure successful application of programs.

1991 - 1997 **Teaching Associate**

Department of Biology, SDSU.
Duties included preparing lectures, assignments and grading.

1996 **Instructor / Research Specialist**

Defense Conversion class.
Developed and taught curriculum on computer analysis in molecular

biology and uses of the internet in science to students converted from defense industry to molecular biology.

1996 National Institute of Health Workshop for CSU faculty and staff. Developed curriculum and taught techniques for polymerase chain reaction (PCR), cloning, and computer analysis of problems in molecular biology.

1989 - 1991 **Research Technician**
Department of Molecular Biology, SDSU.
Responsibilities included carrying out research projects in bacterial cell cycle, cloning, and protein purification, ordering of supplies, lab maintenance and organization, radiation safety officer.

1988 - 1989 **Lab Assistant**
Biological chemistry department, UCLA.
Carried out and assisted in experiments studying clathrin mediated transport in yeast.

**Awards:**

1991 - 1996 National Institute of Health Predoctoral Fellow.

1995 - 1997 ARCS Scholar.

**Presentations and Abstracts:**

April 18 - 25, 1993 Ines Gil, Amy L. Svitil, **Allyn Forsyth**, Vicky G. Newman, Aaron V. Pontsler, and Judith W. Zyskind. DnaA protein effects of ppGpp on *dnaA* gene expression, effects of methylation on DnaA binding to DNA. Poster presentation at the 22nd Annual Keystone symposium on Bacterial Chromosomes.

February 28 -30, 1994 **Allyn Forsyth**, and Judith W. Zyskind. Is Newly Synthesized DnaA Required for Initiation of Replication? Poster presentation at the Lake Arrowhead Genetics Symposium.

June, 1995 **Allyn Forsyth**, Jamie Froelich, and Judith W. Zyskind. Intrinsic bends in the *dnaA* promoter region. Poster presentation at the EMBO Cell Cycle Symposium. Stockholm, Norway.

**Publications and Patents:**

1998 Dalia Y. Kalabat, Jamie M. Froelich, Trung P. Phuong, **R. Allyn Forsyth**, Judith W. Zyskind. 1998. Chitobiase, a new reporter enzyme. BioTechniques 25:1030-35

2001 **U.S. Patent No. 6,228,579**
Title: Method for Identifying Microbial Proliferation Genes
Filing Date: November 14, 1997
Assignee: San Diego State University (Exclusively licensed to Elitra)
Issue Date: May 8, 2001

2002 **R. Allyn Forsyth**, Robert J. Haselbeck, Kari L. Ohlsen, Robert T. Yamamoto, Howard Xu, John D. Trawick, Daniel Wall, Liangsu Wang, Vickie Brown-Driver, Jamie M. Froelich, Kedar G. C., Paula King, Melissa McCarthy, Cheryl Malone, Brian Misiner, David Robbins, Zehui Tan, Zhan-yang Zhu, Grant Carr, Deborah A. Mosca, Carlos Zamudio, J. Gordon Foulkes, and Judith W. Zyskind. A Genome Wide Strategy for the Identification of Essential Genes in the Pathogen, *Staphylococcus aureus*. 2002. Molecular Microbiology 43(6) 1387-1400

2003 **U.S. Patent No. 6,589,738**
Title: Genes Essential for Microbial Proliferation and Antisense Thereto
Filing Date: November 9, 2000
Priority Date: November 9, 1999
Assignee: Elitra Pharmaceuticals, Inc.
Issue Date: July 8, 2003

2003 **U.S. Patent Application Serial No. 09/492,709**
Title: Genes Identified as Essential for Proliferation in *Escherichia coli*
Filing Date: January 27, 2000
Priority Date: January 27, 1999
Assignee: Elitra Pharmaceuticals, Inc.
Status: Allowed

EXHIBIT A

DATE 6/16/99 Batch # 109

**Initial Sensitives**

Plate ID EIM10000118

Scored by P. Bauer

| well # | row | column | score |
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| A1 | 1 | 1 | |
| A2 | 1 | 2 | 1 |
| B1 | 1 | 3 | 2T |
| B2 | 1 | 4 | 0 |
| B3 | 1 | 5 | 1 |
| C2 | 1 | 6 | >6 |
| C3 | 1 | 7 | 1 |
| D2 | 1 | 8 | 2T |
| E1 | 1 | 9 | OT |
| E2 | 1 | 10 | 5 |
| F1 | 1 | 11 | 0 |
| F2 | 1 | 12 | 1T |
| G1 | 1 | 13 | 0 |
| G2 | 1 | 14 | OT |
| H1 | 1 | 15 | 0 |
| H2 | 1 | 16 | 0 |

| well # | row | column | score |
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| A3 | 2 | 1 | 0 |
| A4 | 2 | 2 | 0 |
| B3 | 2 | 3 | 1 |
| B4 | 2 | 4 | 5 |
| C3 | 2 | 5 | 1 |
| C4 | 2 | 6 | 2 |
| D3 | 2 | 7 | 0 |
| D4 | 2 | 8 | 5 |
| E3 | 2 | 9 | 1 |
| E4 | 2 | 10 | >4 |
| F3 | 2 | 11 | 1 |
| F4 | 2 | 12 | 1 |
| G3 | 2 | 13 | 1 |
| G4 | 2 | 14 | 2 |
| H3 | 2 | 15 | 0 |
| H4 | 2 | 16 | >6 |

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| A6 | 3 | 2 | 0 |
| B5 | 3 | 3 | 2 |
| B6 | 3 | 4 | 2 |
| C5 | 3 | 5 | 2 |
| C6 | 3 | 6 | 1 |
| D5 | 3 | 7 | 1 |
| D6 | 3 | 8 | 2 |
| E5 | 3 | 9 | 5 |
| E6 | 3 | 10 | 1 |
| F5 | 3 | 11 | 1 |
| F6 | 3 | 12 | OT |
| G5 | 3 | 13 | 1 |
| [illegible] | 3 | 14 | 2 |

← yidC

| well # | row | column | score |
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| A7 | 1 | 1 | 0 |
| A8 | 1 | 2 | 0 |
| B7 | 1 | 3 | 7 |
| B8 | 1 | 4 | 1 |
| C7 | 1 | 5 | 3T |
| C8 | 1 | 6 | 3 |
| D7 | 1 | 7 | 1 |
| D8 | 1 | 8 | 1 |
| E7 | 1 | 9 | 0 |
| E8 | 1 | 10 | 6 |
| F7 | 1 | 11 | 2 |
| F8 | 1 | 12 | 1 |
| G7 | 1 | 13 | 1 |
| G8 | 1 | 14 | 0 |
| H7 | 1 | 15 | 7T |
| H8 | 1 | 16 | >3 |

| well # | row | column | score |
|---|---|---|---|
| A9 | 2 | 1 | 0 |
| A10 | 2 | 2 | 0 |
| B9 | 2 | 3 | 1 |
| B10 | 2 | 4 | 0 |
| C9 | 2 | 5 | 0 |
| C10 | 2 | 6 | 0 |
| D9 | 2 | 7 | 0 |
| D10 | 2 | 8 | 0 |
| E9 | 2 | 9 | 1 |
| E10 | 2 | 10 | 2 |
| F9 | 2 | 11 | 3 |
| F10 | 2 | 12 | 1 |
| G9 | 2 | 13 | 1 |
| G10 | 2 | 14 | 0 |
| H9 | 2 | 15 | 0 |
| H10 | 2 | 16 | 7 |

STRATAGENE EAGLE EYE II 06/16/99 08:55:48
109 - EIM10000118 #1 06.16.99
IMAGE SIZE (640 × 480 × 8).
INT PERIOD = 0.83 SEC.
ACQUIRED WED JUN. 16 08:55:33 1999.

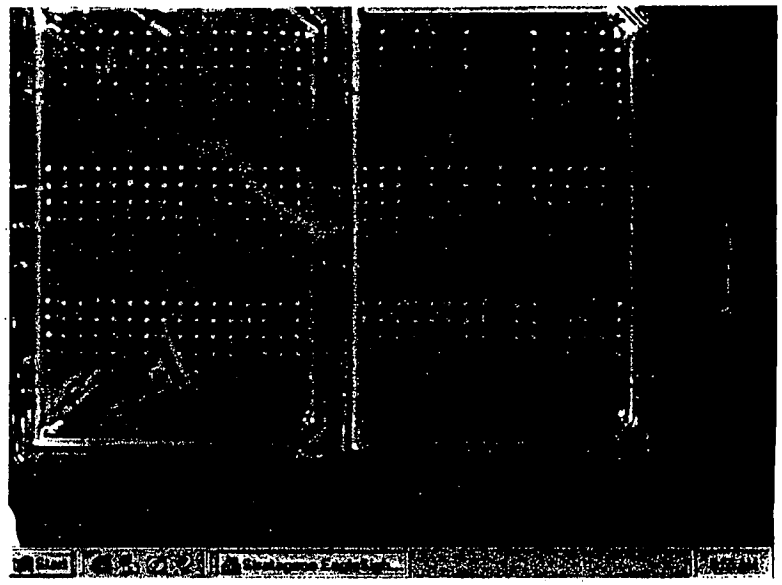

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EXHIBIT B

DATE 8/6/01 Batch # 180 Initial Sensitives Plate ID E1M1...0288 Scored by Ann

| well # | row | column | score |
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| A1 | 1 | 1 | Vector |
| A2 | 1 | 2 | 3T |
| B1 | 1 | 3 | 8 |
| B2 | 1 | 4 | 8 |
| C1 | 1 | 5 | 8 |
| C2 | 1 | 6 | 7 |
| D1 | 1 | 7 | 7 |
| D2 | 1 | 8 | 8 |
| E1 | 1 | 9 | 0t |
| E2 | 1 | 10 | 8 |
| F1 | 1 | 11 | 0 |
| F2 | 1 | 12 | 0t |
| G1 | 1 | 13 | 3t |
| G2 | 1 | 14 | 0t |
| H1 | 1 | 15 | Sterile |
| H2 | 1 | 16 | 8 |
| A3 | 2 | 1 | 3t |
| A4 | 2 | 2 | 2t |
| B3 | 2 | 3 | 7 |
| B4 | 2 | 4 | 0t |
| C3 | 2 | 5 | 0 |
| C4 | 2 | 6 | 8 |
| D3 | 2 | 7 | 8 |
| D4 | 2 | 8 | 8 |
| E3 | 2 | 9 | 7 |
| E4 | 2 | 10 | 0t |
| F3 | 2 | 11 | 0t |
| F4 | 2 | 12 | 0T |
| G3 | 2 | 13 | 8 |
| G4 | 2 | 14 | 8 |
| H3 | 2 | 15 | 3t |
| H4 | 2 | 16 | 0t |
| A5 | 3 | 1 | 1t |
| A6 | 3 | 2 | 6 |
| B5 | 3 | 3 | 0t |
| B6 | 3 | 4 | 2t |
| C5 | 3 | 5 | 1 |
| C6 | 3 | 6 | 8 |
| D5 | 3 | 7 | 7 |
| D6 | 3 | 8 | 7 |
| E5 | 3 | 9 | 2t |
| E6 | 3 | 10 | Vector |
| F5 | | | |
| F6 | | | |
| G5 | | | |
| G6 | | | |
| H5 | | | |
| H6 | | | |

| well # | row | column | score |
|---|---|---|---|
| A7 | 1 | 1 | 7 |
| A8 | 1 | 2 | 8 |
| B7 | 1 | 3 | 8 |
| B8 | 1 | 4 | 5t |
| C7 | 1 | 5 | 7 |
| C8 | 1 | 6 | 6 |
| D7 | 1 | 7 | Vector |
| D8 | 1 | 8 | 0t |
| E7 | 1 | 9 | 8 |
| E8 | 1 | 10 | 8 |
| F7 | 1 | 11 | 8 |
| F8 | 1 | 12 | 0t |
| G7 | 1 | 13 | 2t |
| G8 | 1 | 14 | 0 |
| H7 | 1 | 15 | 8 |
| H8 | 1 | 16 | 8 |
| A9 | 2 | 1 | 8 |
| A10 | 2 | 2 | 8 |
| B9 | 2 | 3 | 6 |
| B10 | 2 | 4 | 6 |
| C9 | 2 | 5 | 7 |
| C10 | 2 | 6 | 8 |
| D9 | 2 | 7 | 3t |
| D10 | 2 | 8 | 8 |
| E9 | 2 | 9 | 4t |
| E10 | 2 | 10 | 4t |
| F9 | 2 | 11 | 8 |
| F10 | 2 | 12 | 8 |
| G9 | 2 | 13 | 0 |
| G10 | 2 | 14 | 8 |
| H9 | 2 | 15 | 8 |
| H10 | 2 | 16 | 8 |
| A11 | | | |
| A12 | | | |
| B11 | | | |
| B12 | | | |
| C11 | | | |
| C12 | | | |
| D11 | | | |
| D12 | | | |
| E11 | | | |
| E12 | | | |
| F11 | | | |
| F12 | | | |
| G11 | | | |
| G12 | | | |
| H11 | | | |
| H12 | | | |

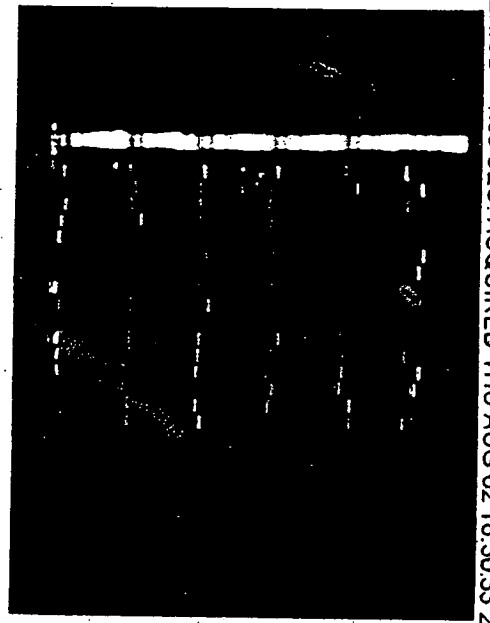
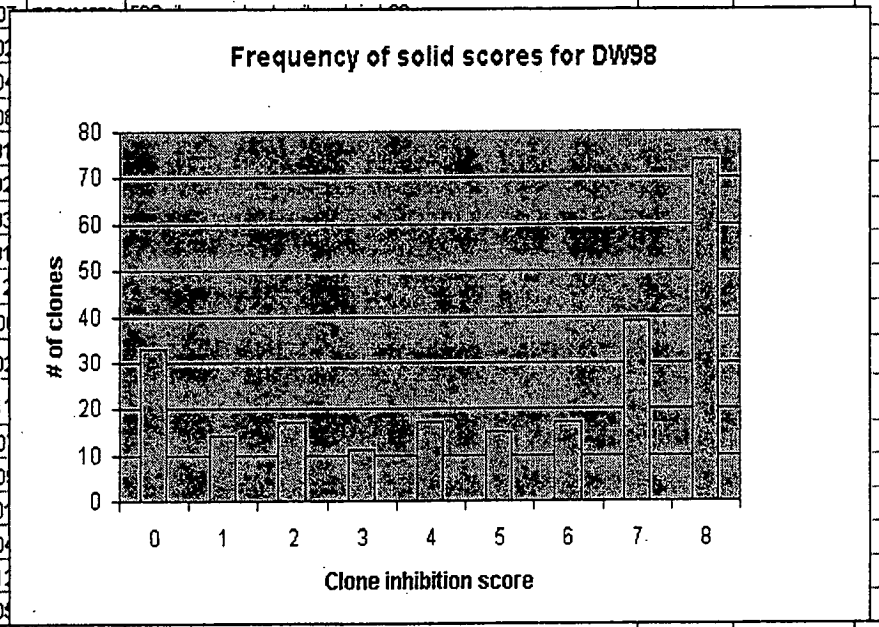


EXHIBIT C

STRATAGENE EAGLE EYE II 08/02/01 18:31:38
e1m10000288
IMAGE SIZE (640 x 480 x 8).
INT PERIOD = 1.33 SEC. ACQUIRED THU AUG 02 18:30:33 2001

BEST AVAILABLE COPY

| Storage Code | Clone ID | Locus | Locus Description | Strain | Plasmid | Library Score | phenotype |
|---|---|---|---|---|---|---|---|
| SEc960 | E1M10000214A07 | | | | | 8 | |
| SEc802 | E1M10000214B02 | | | | | 4 | |
| SEc803 | E1M10000214C04 | | | | | 5 | t |
| SEc961 | E1M10000214D08 | | | | | 6 | |
| SEc804 | E1M10000214E01 | | | | | 6 | |
| SEc805 | E1M10000214E02 | | | | | 3 | |
| SEc806 | E1M10000214E03 | | | | | 0 | T |
| SEc807 | E1M10000214F01 | | | | | 4 | |
| SEc808 | E1M10000214F12 | | | | | 8 | |
| SEc809 | E1M10000214G01 | | | | | 8 | |
| SEc810 | E1M10000214H02 | | | | | 7 | |
| SEc962 | E1M10000214H11 | | | | | 5 | t |
| SEc811 | E1M10000215A01 | | | | | 7 | |
| SEc812 | E1M10000215A05 | | | | | 2 | T |
| SEc813 | E1M10000215B01 | | | | | 8 | |
| SEc814 | E1M10000215B04 | | | | | 2 | T |
| SEc815 | E1M10000215B12 | | | | | 7 | |
| SEc816 | E1M10000215C05 | | | | | 6 | |
| SEc2001 | E1M10000288F12 | ECO101324 | phage superinfection exclusion protein | DW98 | pEPEC3 | | |
| SEc2002 | E1M10000288G06 | ECO100194 | proline tRNA synthetase | DW98 | pEPEC3 | | |
| **SEc2003** | **E1M10000288G07** | **ECO10362** | **60 KD inner-membrane protein** | **DW98** | **pEPEC3** | 2 | t |
| c2004 | E1M10000288H02 | ECO103886 | RNA polymerase, beta prime subunit | DW98 | pEPEC3 | | |
| SEc2005 | E1M10000288H05 | ECO104227 | ORF_o103 | DW98 | pEPEC3 | | |
| SEc2006 | E1M10000289A08 | ECO104091 | priB gene product | DW98 | pEPEC3 | | |
| SEc2007 | E1M10000289B06 | ECO103883 | 50S ribosomal subunit protein L10 | DW98 | pEPEC3 | | |
| SEc2152 | E1M10000289B08 | ECO103624 | ribosomal protein L34 | DW98 | pEPEC3 | | |
| SEc2008 | E1M10000289B11 | ECO103236 | ribosomal protein L16 (rplP) (aa 1-136) | DW98 | pEPEC3 | | |
| SEc2009 | E1M10000289C10 | ECO103885 | RNA polymerase beta subunit | DW98 | pEPEC3 | | |
| SEc2010 | E1M10000289D01 | ECO103237 | ribosomal protein S3 (rpsC) (aa 1-233) | DW98 | pEPEC3 | | |
| SEc2153 | E1M10000289D04 | ECO101475 | FimF protein precursor. | DW98 | pEPEC3 | | |
| SEc2011 | E1M10000289D06 | ECO104213 | regulator for uxu operon | DW98 | pEPEC3 | | |
| SEc2012 | E1M10000289D09 | ECO103885 | RNA polymerase beta subunit | DW98 | pEPEC3 | | |
| SEc2013 | E1M10000289E07 | ECO100095 | cell division; forms circumferential ring; tubulin-like GTP-bindin | DW98 | pEPEC3 | | |




| Score | # of clones | % of Total |
|---|---|---|
| 0 | 33 | 13.92405 |
| 1 | 41 | 17.29958 |
| 2 | 43 | 18.14346 |
| All T,ts | 105 | 44.3038 |

Total clones with scores 237

Total clones on sheet 823

YidC

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EXHIBIT D

# Location of yidC Antisense within the Predicted yidC Operon Structure

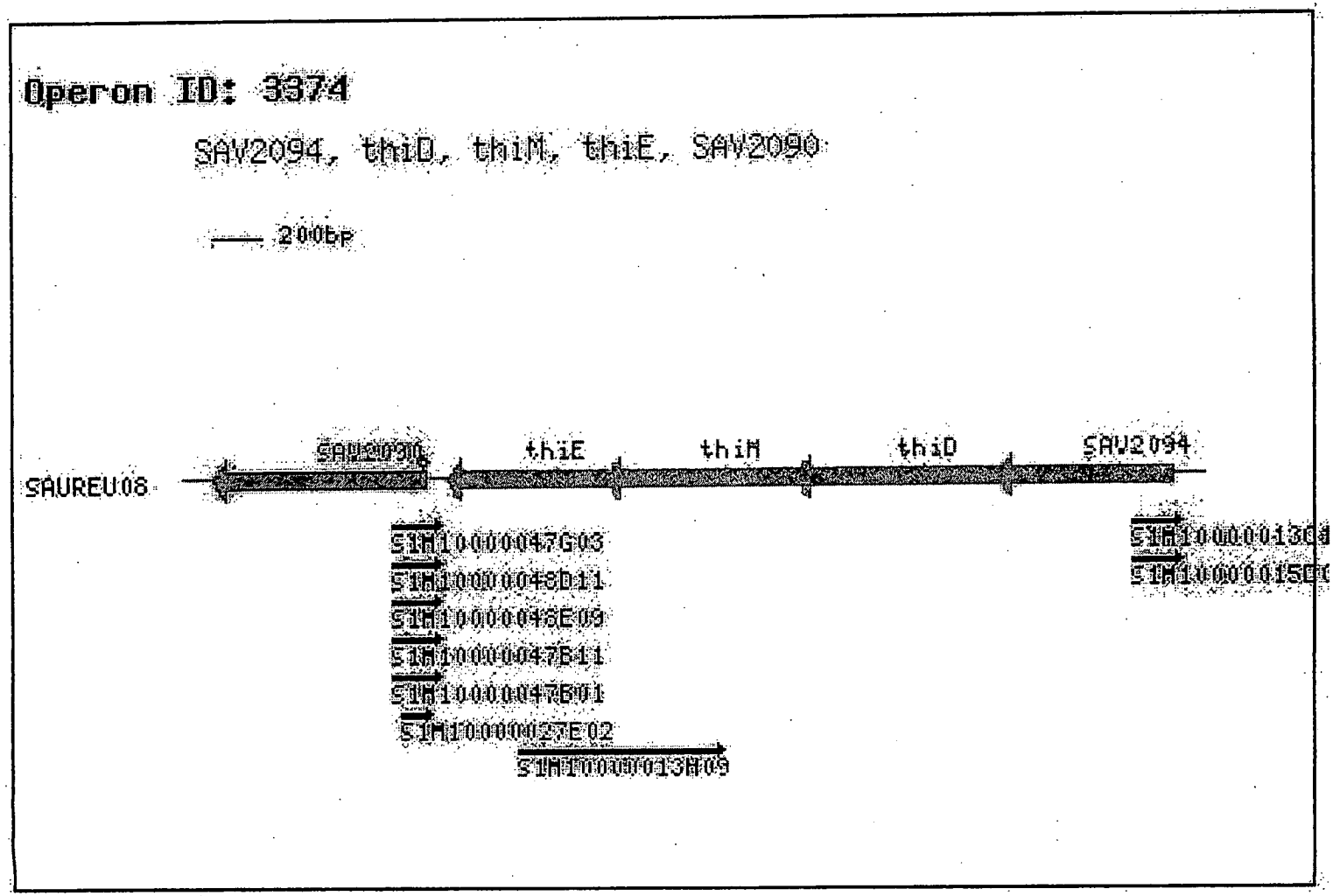





EXHIBIT E

NCBI

Nucleotide Protein formatting BLAST Translations Retrieve results for an RID

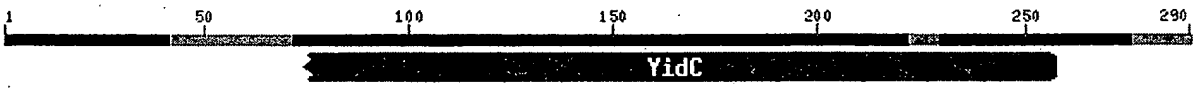
Your request has been successfully submitted and put into the Blast Queue.

**Query** = (290 letters)

---

**Putative conserved domains have been detected, click on the image below for detailed results.**

SAV2090




The request ID is 1059763433-0993-8122

Format! or Reset all

The results are estimated to be ready in 4 seconds but may be done sooner.

Please press "FORMAT!" when you wish to check your results. You may change the formatting options for your result via the form below and press "FORMAT!" again. You may also request results of a different search by entering any other valid request ID to see other recent jobs.

---

**Format**

Show Graphical Overview Linkout Sequence Retrieval NCBI-gi Alignment in HTML

Number of: Descriptions 100 Alignments 50

Alignment view Pairwise

Format for PSI-BLAST with inclusion threshold: 0.005

Limit results by entrez query or select from: (none)

Expect value range:

BEST AVAILABLE COPY

EXHIBIT F

# Genomic insert of yidC Antisense Clone

S1M10000048E09 (S1-843A) Sequence Length: 199

TCCCAACCAATCCAATACATTTTTCATTGGATCTACGAATGTATTG
TAGAAAAACCCACTACGTTTTTCAGGTTTAGAATAGTCACAACCA
GCCAAAAAGACCATAATACCTAAAAATAATGGTAGTAACGCTTTT
TTCTTCATTTTTCCACCTCTATCATTATATTCACATAGGATTTATTC
TATCACATTAATGAGT

EXHIBIT G

# Growth Curve in Response to Xylose Dosing of S1-843A, S. *aureus* RN4220 carrying yidC antisense

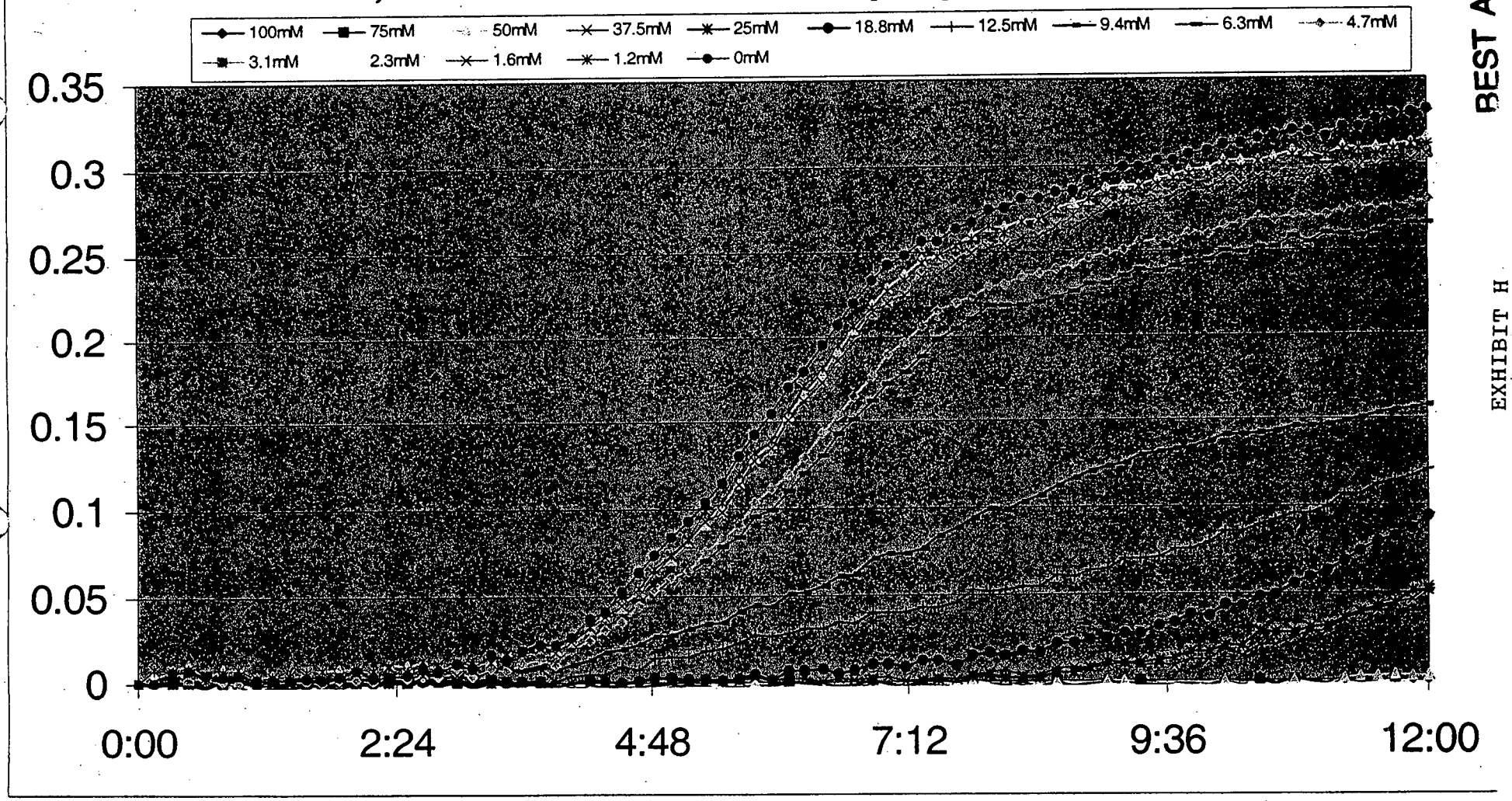



EXHIBIT H

# Xylose Dose Response of S1-843A at an $OD_{600} = 0.1$

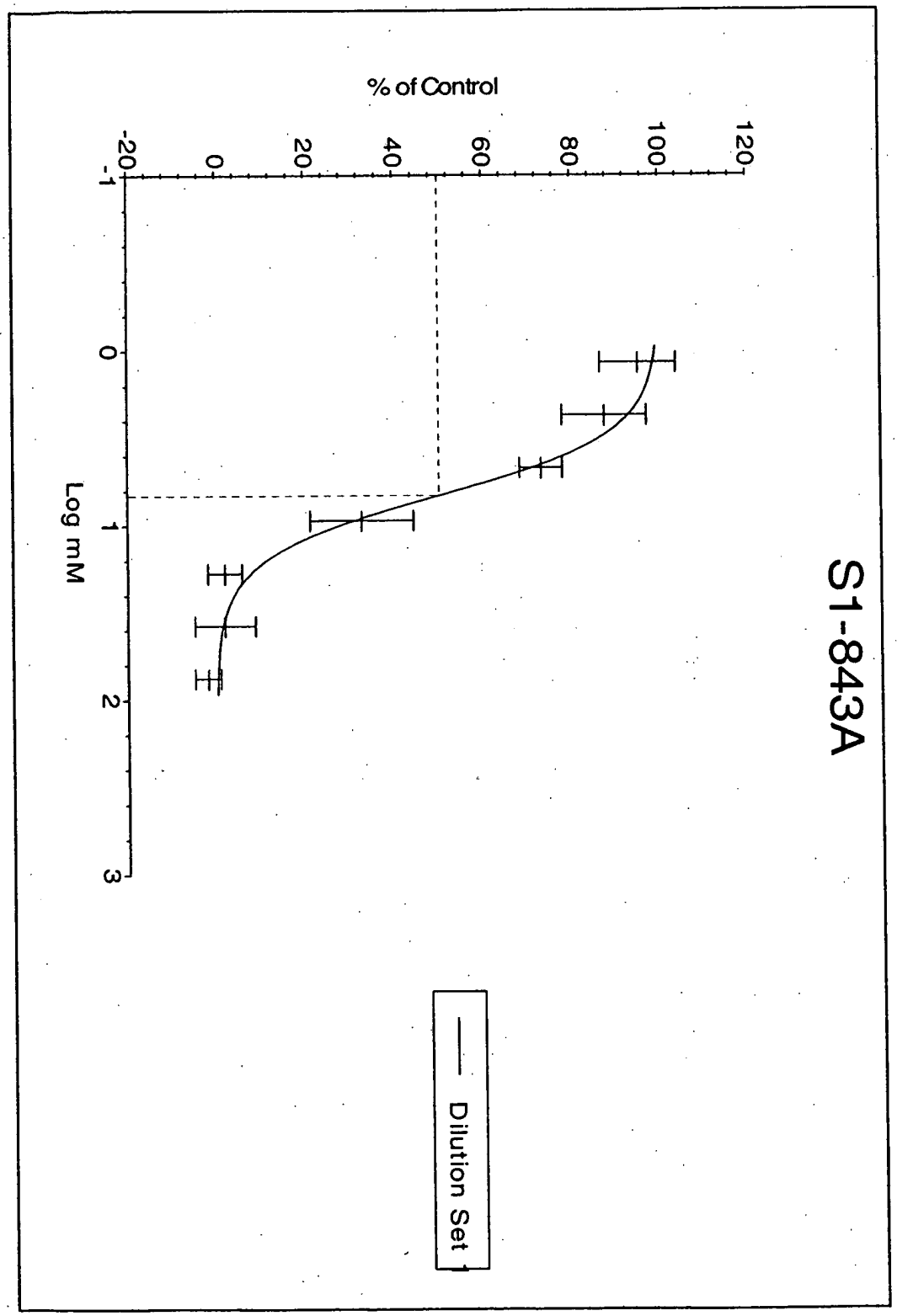



EXHIBIT I